University of Washington
INVENTION DISCLOSURE (FORM ID-1)

Use this form to disclose inventions to the University's Office of Technology Transfer, Box 354810. Prompt disclosure allows the University to secure intellectual property rights as appropriate and to fulfill obligations it may have to external sponsors of research.

Project Number (Include if you have been assigned an OTT project number) 2606 PR

1. Title of Invention

A novel strategy to target anti-HIV drugs to lymphnodes and lymphoid cells

2. Description (briefly describe general nature, primary functions, and areas of principal use)

The goal of this invention is to improve our ability to reduce virus found in latent reservoirs. Using lipid-drug complexes of about 50 nm, we were able to achieve selective accumulation of an anti-HIV drug in lymph nodes.

3. Funding Source(s) Was work leading to the invention supported by:

A. Internal allocations from:
- Graduate School Fund? Yes____ No X
- Royalty Research Fund? Yes____ No X
- Washington Technology Center? Yes____ No X

Project Title Kinetics and Mechanisms of Mother to Fetus HIV Transmission

B. Federal and/or External Grant or Contract awards: Yes X No____

Grant/Contract #s NIH HL 56548 + AI 31854 (per R. Ho/tcw 8/1/2004) AI 52663

4. Government Employee(s) Please list any inventors associated with the V.A. Medical Center.

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OTT Date Received: OTT Disclosure Number Assigned: 3342 DL rev 09-95

MAR 22 2000
3/23/00

EXHIBIT 2

Disclosure #: 3342

University of Washington
INVENTION DISCLOSURE SUPPLEMENT
(FORM ID-2)

The information requested on this form documents the development history of an invention previously disclosed to the Office of Technology Transfer. This information is necessary for the University to evaluate the invention for possible patent rights and commercial applications. **Please complete both sides of the form. Attach additional documents as needed.**

Project Number (Include if you've been assigned an OTT Project Number) 2606 PR

1. Invention History. Where a date is not well documented, indicate that the date is approximate using a phrase such as "on or about" or give a span of time in which the event took place. For locations on campus, use "UW"; for others, provide the name of city and state.

Stage of Inventive Activity	Date	Location
Initial idea (conception)	3/1999	H272H
First verbal description (public or private) to others	6/1999	H272H
Invention development records, notes, drawings (evidence of diligence)	7/1999	H264
First description of complete invention, oral or written (first constructive reduction to practice)	8/1999	H264
Any sale or public use of the invention in the United States		

Note: **"Sale"** means (generally) "on sale," including any offer to sell or any actual sale to a purchaser, whether or not such "sale" activity was secret or non-secret. **"Public Use"** means (generally) use of the invention by the inventor in public, or used by the public. Other secret or non-secret uses of the invention by other than the inventor may also qualify as public use.

2. CV and External Funding. Provide a current copy of each inventor's CV (listing all papers each inventor has authored or co-authored). List and provide **copies of any funded or unfunded grant proposals** related to the invention, and state the funding status for each.

HL56548
AI31854

3. Publications. List any reports, abstracts, papers, or theses related to the invention that have been published or are in preparation. Include **two complete copies of** the first publication that describes the invention, and **provide date of publication or intended publication.**

See the attached proposal submitted in confidence to NIH for funding

4. References. List any references, issued patents, patent applications, review articles, or other publications that pertain to the invention. Include copies if available.

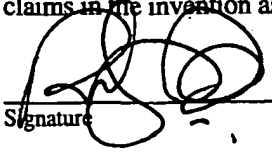
5. Key Words (for on-line patent and literature searches).

anti-HIV drug/ lymph node targeting/ lipid drug interactions / high degree of association

Please notify OTT if you become aware of any additional publications, patents, or other references pertaining to the invention.

(continued on reverse)

6. Inventors. Include those individuals who have made creative contributions to the invention. For patents, determination of inventors depends on the particulars of the invention specification and allowed claims in the invention as described in the patent application.

Signature  Date 3/16/2000	Signature Date
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Address (Home)	Address (Home)
Phone: Work Home	Phone: Work Home
Social Security No. - -	Social Security No. - -
Citizenship	Citizenship

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A novel strategy to enhance anti-HIV drug accumulation to lymphoid tissues and cells

I. Specific Aims

The long-term goal of this program is to develop a mean to reduce HIV found in latent reservoirs. While the additional reservoirs outside of lymphatic are remained elusive, it is clear that a significant fraction of latent virus is found in visceral (e.g., mesentery) lymph nodes, in addition to peripheral nodes that can be readily accessed by biopsy procedures in humans. The use of HIV or SIV infected primate will provide a mean to systematically evaluate drug and virus concentrations simultaneously at both peripheral and visceral (i.e., mesentery) nodes in an attempt to correlate drug concentration in tissue to virus load. The use of lipid associated drug complexes to achieve higher concentration of drugs in lymph nodes would allow us to discern whether increasing local drug concentration would further reduce the virus load in the latent reservoir.
Hence, with SIV or HIV-2- infected macaques and indinavir as a model anti-HIV drug, the specific aims of this proposal are designed to test the following hypotheses

Hypothesis 1: Drug concentrations in lymph nodes can be enhanced by delivery of a model drug, indinavir in lipid-associated form, in comparison to free drug formulation.

Hypothesis 2: Enhanced drug accumulation in lymph nodes further reduces the seemingly constant virus load in lymphoid tissues achieved with free drug administration.

II. Background

HIV infection continues to be a major health problem in the United States. A number of anti-HIV drug combinations (often referred to as HAART, highly active anti-retroviral therapy) that attack the virus by mechanisms including inhibition of reverse transcriptases (selective for either nucleoside or no-nucleoside), and viral proteases have maximally decreased the virus concentration in blood of HIV infected patients on chronic therapy. However, a significant proportion of virus in the HIV infected individuals are found in lymphoid tissues and the HIV concentration in blood do not predict the virus concentration in lymphoid tissues. Data from recent reports (Hockett et al., 1999 J exp med 189, 1545; Pantaleo et al.,1993 Nature 362:255; Pantaleo et al., 1991 Pro Natl Acad Sci USA 88:9838) demonstrated that viral RNA concentrations in lymph nodes remained

relatively constant over the course of HAART in HIV infected patients, despite the low or undetectable levels of HIV in blood or plasma. These data suggest that either the virus found the lymph nodes are resistant to the drug therapy or insufficient exposure of drug to this tissue. However viruses isolated from lymphoid tissues and plasma are equally sensitive to anti-HIV drugs, suggesting the possibility that insufficient drug exposure to lymphoid tissue and cell may be the key to eliminate residual viruses. Therefore, if one can devise a strategy to improve delivery of drugs to lymph nodes at higher concentrations for longer duration, it is likely that virus concentration in lymph node can be greatly reduced.

We have taken a novel approach to a drug carrier design that will likely increase delivery of anti-HIV drug to lymph nodes at higher concentrations for a prolonged period. Lipid-drug particles of 50-80 nm in diameter that are stable in biologic fluid may enhance drug localization in cells and tissues of lymph nodes. While dendritic cells are less efficient than macrophages in particle uptake, both of these cells, considered to be sanctuary of HIV, can uptake the small lipidic particles more efficiently than fluid uptake or pinocytosis. Lipid vesicles or liposomes, with spherical shaped enclosed lipid bilayers with a 50 nm to several micro meters in diameters size have been used successfully to enhance delivery of highly potent anti-fugal and anti-tumor drugs. When small liposomes of about 50-80 nm are given by either IM or SC route or directly injected into the lymphatics, the small liposomes are distributed first into lymphatic, and subsequently, a significant of them are trapped at the draining lymph nodes (Kim and Han 1995, J Microencap 12:437; Himle 1997 Hybridoma 16:127). If one can either encapsulate or incorporate an anti-HIV drug into lipid (or liposome) bilayers with high efficiency and stability, the lipid associated drugs can be delivered to lymph nodes with great efficiency in vivo. This will overcome insufficient anti-HIV drug exposure in lymph nodes in clearing the virus dwell in lymph nodes.

In search of the model drug to be tested, we found that anti-HIV drug indinavir would be an ideal drug for incorporating it into lipid bilayer of liposomes at neutral pH (pH=7) for targeting it to lymph nodes. Indinavir exhibit a low aqueous solubility (but high lipid solubility) at pH 7, and a high aqueous solubility at pH 3.0. (The currently available oral indinavir dosage form is formulated with citrate buffer to achieve pH value around 3 to enhance solubility of indinavir for gut absroption). Membrane associated drug will provide the stability of the lipid-drug complex in biological milieu before accumulating in the lymph nodes. As a result, the liposomes loaded with drug molecules can be either taken up by the lymphoid cells and/or provide sustained presence of drug as liposome are gradually metabolized by the lipases found at cell surfaces within lymphoid tissues. This approach may (1) provide much higher concentration of drugs in lymph

nodes that cannot be achieved with free drug administration, and (2) increase intracellular concentration of drug in cells of lymph nodes and systemic circulation for the HIV infected cells that uptake 50-80nm particles. As a result, this strategy may likely to further reduce HIV replication in lymphoid tissues.

III. Preliminary Studies

Effect of pH on the ability of indinavir to associate to lipid bilayer

Using indinavir with about 1000 fold decrease in aqueous solubility differences between pH 3 and pH7 (Lin et al. 1995 Drug Metabolism and Disposition 23:730), we determined the effect of pH on the ability of liposomes to encapsulate or incorporate the drug. At lipid-to-drug ratio of 5:1 (m/m), practically all (80-90%) the drugs in the preparation were found to be associated with liposome at pH-7 (Figure 1). At lower pH value (i.e., 3) where aqueous solubility of drug is higher, we found much lower degree (<30%) of drug incorporated into liposomes. Since the physiological pH value is 7.4 and biological fluids are highly buffered, these lipid-associated drugs are expected to remain stable. Hence, we use the lipid-indinavir complex formed and maintained at pH 7 for the subsequent pharmacokinetic study.

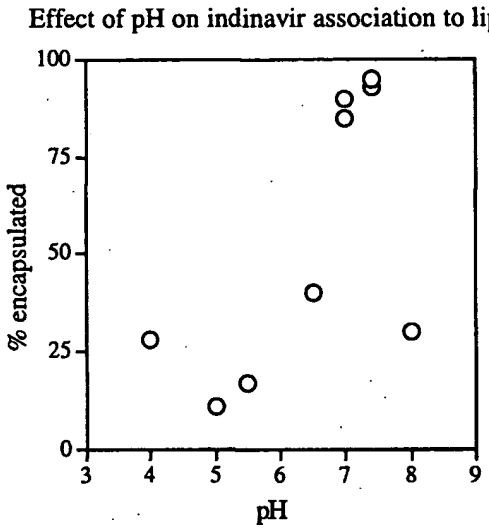

Figure 1: Effects of pH on incorporation of indinavir to liposomes. With lipids containing phosphatidyl choline (egg) : cholesterol (3:1,m/m) and lipid to indinavir 5:1 (m/m), small unilamellar liposomes were prepared with buffer at indicated pH value. They were sonicated to achieve 50-80 nm in diameter. Subsequently, the % lipid-association was determined by separating free from lipid-associated drug by size-exclusion column chromatography. Data expressed were mean of duplicates preparations for indicated pH value.

Plasma time course profile of free vs. lipid associated indinavir in macaques

We prepared two doses of liposome formulated indinavir to compare with that of free drug. Young adult (~5-6 kg) macaques administered (SC) with indinavir suspension in DMSO and phosphate buffer produced a plasma drug concentration peak at about 0.5-1 hr and rapidly cleared to below detection level by 6 hr (Figure 2). In contrast, liposome formulated indinavir produced more than ten-fold lower peak plasma concentration and sustained plasma level beyond 10 hrs. In fact, when a second dose was given after 30 day

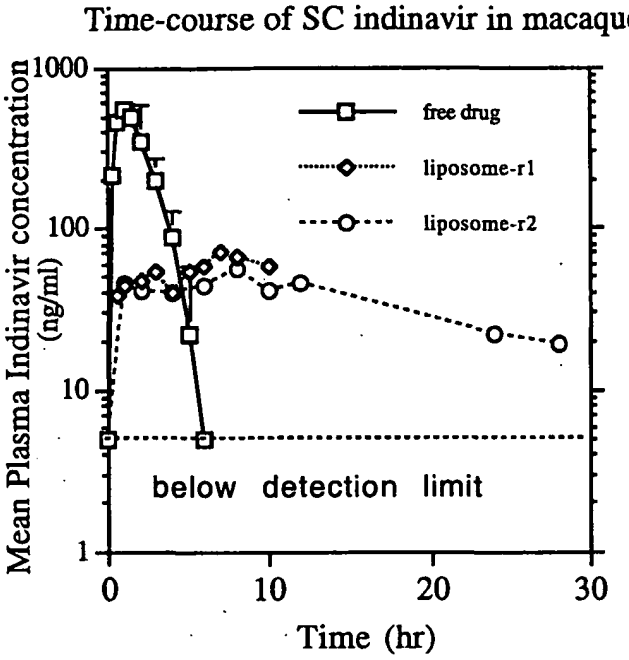

Figure 2: Plasma time course comparison of macaques subcutaneously administered with free vs. liposome formulated indinavir. Young adult macaques were sub utaneously injected with either free or liposome formulated indinavir at 10mg/kg dose and plasma drug concentrations were determined with a HPLC assay. Data expressed were mean ± SD for animals injected with free (□, n=4) and liposome formulated indinavir (○,◇, n=2)

wash-out period, we found that a significant amount of drug (>20ng/ml) remained in plasma beyond 24 hr (Figure 2). Because this animal did not exhibit a detectable peripheral lymph node, and our budget constraint did not justify euthanizing the animal for visceral (i.e., mesentery and other) lymph nodes collection, the effect of lipid association on indinavir ability to accumulate in lymph nodes remained elusive. We hope to collect this critical information with the propose fund and resources to determine plasma drug concentration in relation to lymph node drug concentration in animals administered with free and liposome formulated indinavir.

Evaluation of cytology at injection sites.

Pathological analysis of biopsy collected from the injection sites indicated that liposome carrier alone did not induce local lymphocytosis. When lipid formulated or free indinavir were subcutaneously administered, there were drug related local inflammation that

resolved soon after drug is cleared from injection site. With liposome formulated indinavir, but not with free liposomes, we also found lymphcytosis in the proximity, suggesting that liposome associated indinavir may provide a mean to target indinavir to lymphocytes.

IV. Research Design & Methods

Hypothesis 1: Drug concentrations in lymph nodes can be enhanced by delivery of a model drug, indinavir in lipid-associated form, in comparison to free drug formulation

While the direct comparison of an anti-HIV drug in lymph node and blood is not yet available, it is believed to be less than unity or one after oral or IV administration. In other words, a very small fraction of total dose will be distributed to lymphoid tissues. Even with direct lymphatic delivery of free drug, it will rapidly redistributed into the blood and eventually eliminated. Taking advantage of the ability of small particles (50-80 nm in diameter) to localize in lymph nodes throughout the lymphatic system, and the ability of indinavir to stably associate with the lipid (liposome) particles, we anticipate that administration of lipid-associated indinavir will exhibit drug concentrations in lymph node higher than that in blood. As dosing with free indinavir drop below detectable level by 6 hrs and dosing with lipid-associated inidnavir remained detectable up to 28 hr, we propose to evaluate drug concentration in the (peripheral and visceral) lymph nodes at early (3 hr) and late (5-6 hr) time points. Because the slower rate of distribution and longer presence of drug in plasma for animals administered with lipid-associated drug, we propose to determine drug concentration in lymph nodes at one additional time point, 24 hr, only for animals treated with lipid associated drug. With at single dose of 10mg/kg either in lipid associated or free form, we will use 2 animals per time point: two time points for free drug treatment, and three time point for liposome-associated drug treatment. For the proposed experiment, we will need 10 animals.

With the proposed experiment, we will determine whether or not (1) drug concentration in lymph nodes is lower than that achieved in blood after free drug administration, (2) drug formulated as small lipidic-particles increase the drug concentrations in lymph nodes, and (3) similar distribution of anti-HIV drug, indinavir in peripheral and visceral lymph nodes is observed after free and liposome-formulated drug. If much lower drug concentration in lymph node is observed, it is possible that limited availability of drug to lymph node may be one of the mechanisms leading to residual virus found in lymph nodes.

Hypothesis 2: Enhanced drug accumulation in lymph nodes further reduces the seemingly constant virus load in lymphoid tissues achieved with free drug administration.

While it remained to be shown directly, prediction based on the mass action of drug distribution from blood to lymph nodes, and data collected from drug (with no direct ligand-receptor interactions in the tissue) distribution studies indicate that most drugs produce lower concentration or exposure in tissues than in blood. With the sustained plasma profile achieved (Figure 2), and the anticipated slower distribution but higher degree of particle accumulation of lipid-indinavir, we will determine the effect of increase magnitude and duration of drug in lymph nodes further reduce the virus presence in peripheral and visceral lymph nodes. We propose to use macaques infected with highly pathogenic HIV-2_{287} or $SHIV_{89.6p}$ that produce intense viremic phase followed by an almost complete decline in CD4+ T cell (below 5%). At this stage, very low concentration of virus (< 1-10/10^6 virus infected cells or <10^3/ml plasma) is detected in blood. We will treat these macaques for 1 week with AZT (25mg/kg). A detectable amount (2-10 infected/10^6 cell s) of virus was found in lymph nodes at this post-viremic phase (about 4-6 weeks post infection). We will compare the effect of free vs. lipid-associated indinavir on their ability to further reduce the virus load in peripheral and visceral lymph nodes. To collect preliminary data to test this hypothesis, we will need a minimum of 10 animals (5 animals each for free and lipid-associated inidnavir treatment). We will treat the animals for 7 days and collect the tissue for drug and virus evaluation 48 hrs after the final dose of inidnavir. The dosing scheme proposed for free (TID) and lipid associated drug (QD) at 20mg/kg/day is based on the time-course drug concentration profile (Figure 2) and virus sensitivity (IC_{50}= 0.04μM for HIV-2_{287} and $SHIV_{89.6p}$) of inidnavir.

V. Relevance

We must emphasize that the current proposal is designed to collect data on elucidating likely mechanisms leading to virus found in lymph nodes, and a proven strategy to enhance delivery of anti-HIV agents, cytokines and drugs to these privilege sites in reducing the residual virus load. As visceral lymph nodes are thought to be the major tissue sites that harbor the HIV, our ability to direct the drug and cytokines to cells in lymphatic will be crucial for eradicating virus found in these privilege sites with limited drug access. A successful development of this strategy may permit directing cytokines (e.g., IL-2) to activate the latently infected cells to produce virus allowing anti-HIV

inhibitors to control the virus replication by eliminating these latently infected cells in the process. Additional strategies can be developed to deliver both cytokines and anti-HIV drug combinations based on this principle.

Added notes:

Recently, we have administered two additional infant macaques with 10mg/kg lipid-associated indinavir and harvested inguinal lymphnodes at 24 and 28 hrs. Drug concentration in blood as well as lymph nodes were measured. We found that at 24 and 28 hrs, indinavir was detectable in plasma and lymph nodes of both animals. Our historical data indicate that macaques given free drug in DMSO exhibit no detectable indinavir in plasma or lymphnodes beyound 8 hr and lymphnode to plasma drug ration never exceed one. In contrast, we found that the lymphnode to plasma ratio at these time point were 3.4 and 7.1 for the two animals. These preliminary data suggest that lipid associated inidnavir may provide the enhance lymphnode accumulation of indinavir to the level that cannot be achieved by free drug administration. In addition, it may also increase resident time in plasma, as demonstrated above